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Intermediate Filaments: A Historical Perspective

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Abstract

Intracellular protein filaments intermediate in size between actin microfilaments and microtubules are composed of a surprising variety of tissue specific proteins commonly interconnected with other filamentous systems for mechanical stability and decorated by a variety of proteins that provide specialized functions. The sequence conservation of the coiled-coil, alpha-helical structure responsible for polymerization into individual 10 nm filaments defines the classification of intermediate filament proteins into a large gene family. Individual filaments further assemble into bundles and branched cytoskeletons visible in the light microscope. However, it is the diversity of the variable terminal domains that likely contributes most to different functions. The search for the functions of intermediate filament proteins has led to discoveries of roles in diseases of the skin, heart, muscle, liver, brain, adipose tissues and even premature aging. The diversity of uses of intermediate filaments as structural elements and scaffolds for organizing the distribution of decorating molecules contrasts with other cytoskeletal elements. This review is an attempt to provide some recollection of how such a diverse field emerged and changed over about 30 years.

Introduction

Intermediate filaments are approximately 10 nm diameter, intermediate in size between actin microfilaments and microtubules. These filaments are coded for by 65 human genes defining five classes of intermediate filament proteins and two more distal related beaded lens filament proteins [1] (Table 1). The first two classes are the type I and II keratins that represent 54 subunits of these obligate heteropolymers that define epithelial tissues and hair [2]. All 28 type I keratins are clustered on human chromosome 17 except for keratin 18 that is located at one end of the type II keratin cluster on chromosome 12. All 26 type II keratins and K18 are located on human chromosome 12. The third class includes the homopolymeric filament proteins vimentin [3], desmin [4], glial fibrillary acidic protein (GFAP) [5] and peripherin [6]. The three neurofilament subunits NF-L, NF-M and NF-H, [7] established the fourth class of IF proteins to which were added nestin [8], alpha internexin [9], syncoilin [10] and synemin [11]. Up to five nuclear lamins, coded for by three genes, compose the nuclear lamina and define the fifth class of IF proteins.

The history of intermediate filaments (IF) could be considered to have divergent beginnings, a shared common discovery phase and an interesting and divergent future, something like the structure of intermediate filament proteins. This review is a personal, and undoubtedly (but not intentionally) biased review of some of the highlights of discoveries about IFs that has led to our present understanding and continued curiosity about this very large family of proteins.

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Over the last 30 years, many reviews of all aspects of IF have been published. Historically, some of the most highly cited have been provided by Lazarides [12], Fuchs and Weber [13] and Steinert and Roop [14] and more recently Fuchs and Cleveland [15]. In addition, reviews of IF proteins in disease [16], the dynamic properties of IF [17,18], function in neuronal development and disease [19], keratin diseases [20], IF structure [21–23], lamin diseases [24], IF function in Caenorhabditis elegans [25], IF as signaling platforms [26], and this issue of this journal are all highly recommended. One important early review by Elias Lazarides was titled, “Intermediate filaments as mechanical integrators of cellular space” [27]. This phrase cleverly described the dramatic, filamentous patterns of proteins that had not yet been linked to specific molecules or functions. Indeed the identification of proteins that linked the IF networks to the actin network, membrane anchoring platforms and a variety of decorating proteins has provided the proof of this early speculation.

X-rays and sheep ranches of Australia

IFs in the form of specialized hair keratins have been the target of attention even before the invention of science. The advancements of understanding the composition, structure and function of IF are attributable to a significant degree to the application of new tools or methods that stimulated flurries of scientific activity. It was the application of x-ray diffraction and crystal structure theory that led William T Astbury in 1932 to publish the first data of a periodic structure of keratin [28]. The possibility of determining molecular structure of a highly ordered hard keratin polymer was stimulated by the interpretation of alpha keratin diffraction patterns in light of Linus Pauling’s alpha helix model. This led to the prediction of the alpha helical coiled coil structure by Francis Crick in 1952 [29,30]. The challenge of interpreting the diffraction pattern of alpha-keratin was pursued through the 1990s. However, it was not until individual proteins were purified and reconstituted that the probability of solving the atomic structure of IF was greatly advanced. The problem of the molecular structure of keratins stimulated the attention of physical biochemists including Australians, George Rogers and his student, Peter Steinert, who started as a wool biochemist and spent his career investigating both the physical and biological aspects of keratins and skin until his passing in 2003. It was his discovery of the polymerization of IFs from denatured, soluble keratin by dialysis into lower ionic solutions [31] that provided the physical assay for assessing the protein subunit requirements necessary for filament formation. This permitted the evaluation by electron microscopy of the heteropolymeric requirements of keratins and neurofilaments and the homopolymeric filaments of other types of IFs. In the 54 years since Crick’s prediction of coiled coil structure of keratins, the problem of an atomic model of the polymerization of IFs into defined diameter filaments has remained a fascinating challenge that may be nearing a solution, ironically, by methods that force parts of the filamentous proteins to adopt crystalline packing arrangements [32–34]. While the structure of IF is the defining characteristic of the gene family and the driving force of the evolutionary conservation of primary structure, the IF structure is used in multiple ways such as structural elements or internal scaffolds for the docking of regulatory proteins.

Electron microscopes and Chilean fishing boats

IFs became real to many when they were visualized by electron microscopy. The electron microscope was invented in 1931 and was available commercially in 1939. However methods for preparation of biological specimens for examination in the electron microscope were not commonly embraced until the 1950s with improved preparation methods and scanning technology. With the widespread examination of cells with electron microscopy, it is likely that a number of investigators in the 1960’s saw IFs in different tissues but failed to recognize them as distinct from smaller actin and larger microtubules. Holtzer and colleagues named IFs during their investigations of muscle [35].

The recognition of filamentous proteins in neuronal axons is likely as old as silver staining for light microscopy. However, the recognition of neurofilaments of about 10 nm in diameter may have started in the sea off the coast of Chile where the ferocious Humboldt or jumbo squid, *Dosidicus gigas*, was harvested. The large size of the squid giant axon provided a source for axoplasm, axon cell contents, that was investigated by electron microscopy and biochemical methods. While the sensitivity of neurofilaments to calcium dependent proteolysis, and differences between human and other species neurofilament proteins muddled the exact protein composition of neurofilaments for several years, clear microscopic evidence was presented for abundant filaments intermediate in size between actin and microtubules [36].

Gene analysis of NF started when Julien cloned the neurofilament subunits [37]. Julien and Cleveland initiated a careful series of gain and loss of function experiments in mice [19]. The abundance of neurofilaments led to the hypothesis that they might control the caliber of axons, a key characteristic that governs signal conduction velocity. Transgenic over expression, targeted gene mutations and domain switching studies confirmed that axon radial growth was dependent on NF, but surprisingly, it was the C-terminal domains of NF-M that were key for receiving signals from the surrounding glial cells that influence radial diameter [38] much more than the very large repeated arrays of phosphorylation sites of the C-terminal domain of NF-H [39–42]. The influence of NF on axon caliber was due to a more subtle mechanism than filling cytoplasmic space. Julien, Cleveland and others observed effects of over expression of NF subunits and mutant forms on neuron health similar to human disease [43–45]. Mutation of the NF-L subunit is associated with Charcot-Marie-Tooth disease [46]. NF mutations also may be a risk factor but not a primary cause of ALS [47].

Discovery and coalescence

While seeing IFs convinced investigators of a third cellular filament system, it was the variety of different IF proteins, in contrast to microfilament and microtubules, that stimulated great attention. The many unique proteins responsible for apparently similar sized filaments focused the efforts of many investigators throughout the 1970s and early 1980s. Glial fibrillary acidic protein (GFAP) was the first IF protein to be recognized by a specific antibody [5] and to be used as a reliable marker of glial and astrocyte cell identity. While GFAP was considered a reliable marker of astrocytes, current investigations have implicated GFAP positive cells as candidate source of multipotent neuronal derivatives [48]. Indeed, the use of IF proteins as markers of specific types of cells is arguably the most important, and certainly the most common, application of IF research. Lazarides and Weber, two major figures of IF discovery preceded their IF research with studies of the actin cytoskeleton using specific antibodies to reveal actin filament pattern in cells [49]. It was eight years after Holtzer's recognition of muscle IF by electron microscopy before the responsible muscle protein, desmin, was purified [4]. Purifying an IF protein, generating specific antibodies and demonstrating its capability to form IF became the essential paradigm.

1978 was a good year for IF. Hynes' laboratory purified the fibroblast 58kD IF protein, generated distinguishing antibodies and contrasted it with the behavior of actin and microtubule networks [50]. However, it was Franke and colleagues who also purified it and further named the fibroblast IF protein **vimentin** later the same year [51]. The productive collaborations between Franke, Osborn and Weber demonstrated the power of cell type specific IF antibody tools [51]. In addition, Liem and colleagues [52] resolved ambiguity about the sizes of neurofilament subunits and GFAP. By the end of 1978 the major IF proteins, neurofilaments, GFAP, desmin, vimentin and keratins were all recognized, though sorting out the many keratins and the interaction of the three neurofilament subunits had just begun.

In contrast to the use of purified protein antigens, at least two sets of investigators, separated by the Atlantic Ocean, recognized the implications of cross reaction of keratin antisera with a variety of different cultured epithelial cells and tissues. Howard Green at MIT headed the first group and Werner Franke the second. Green, the inventor of 3T3 cells, was responsible for determining the conditions that permitted the cultivation and differentiation of human epidermal keratinocytes. Sun and Green used the cross reaction of human epidermal keratin antibody with a variety of cultured epithelial cell types to identify intracellular keratins and later contributed widely used, broadly reactive, monoclonal keratin antibodies [53]. The culture system permitted the analysis of the requirements for proliferation and differentiation of keratinocytes. This discovery eventually also resulted in the production of keratinocytes for patient transplantation.

The groups in Boston and Heidelberg took different and complementary investigative strategies on keratins. In Green's laboratory, Elaine Fuchs with the initial help of Don Cleveland adopted a molecular biology paradigm to sort out the epidermal keratin proteins by cloning the RNAs and genes, and thus providing definitive criteria of identity. Remarkable progress was made in defining the molecular changes of keratin proteins, RNAs and genes [54–58], that set the stage for discovering the molecular basis for the first genetic disease caused by mutations in intermediate filament proteins. The family tree of major contributors on keratin IF continued from the Green lab to the Fuchs laboratory and then to Pierre Coulombe, who continued to explore the physical and functional roles of keratins [16,18,59].

Mean while, Werner Franke and Klaus Weber recognized that the cross reaction of multiple keratin antibodies with epithelial cells must reflect significant evolutionary and structural conservation of a family of cytoskeletal proteins related to hard keratins [60]. Franke's group surveyed the expression of keratin proteins in an amazing diversity of cell types, animals, developmental and pathological states, using two dimensional gel analysis of proteins and a variety of antibodies to eventually produce a catalog of the expression patterns of keratin proteins that may be the most highly cited IF paper [61]. These comparative studies demonstrated a clear distinction between keratins of simple or single layered, epithelial tissues, multilayered or squamous epithelia and hair or hard keratins. Further recognition of the two major protein groups of acidic (Type I) and basic (Type II) keratin subunits and the requirement for at least one of each type to reconstitute 10 nm filaments [31,62] fit well with the two gene families identified by Fuchs and coworkers [63]. In 1982 Weber discovered the IF tripartite structure of a central alpha-helical domain flanked by non-helical terminal domains by sequencing the desmin protein [64]. From 1978 to 1988, Franke published 112 papers on keratins as well as more than that number on other topics. During this period, the Franke laboratory produced many current, independent IF investigators including Jose Jorcano, Harald Herrmann, Thomas Magin, Rudolf Leube and Roland Moll.

IF and stem cells

In the 1970s and early 1980s, the paths to IFs merged from keratin molecular structure (Steinert, Aebi), epidermal biology (Green, Fuchs, and Roop) and the cytoskeleton (Lazarides, Weber, Franke, Goldman). However, additional investigators arrived at IF via studies of early embryonic development. To generate markers of the earliest differentiated cell types of the mouse embryo, Brulet, Kemler and Jacob made monoclonal antibodies against undifferentiated teratocarcinoma cells and cytoskeletal fractions of differentiated derivatives [65,66]. Oshima purified mouse K8 (Endo A, cytokeratin A) and K18 (EndoB, cytokeratin D) as markers of the differentiation of mouse embryonal carcinoma cells and in combination with two-dimensional gel electrophoresis found that these proteins were the antigens for two of the monoclonal antibodies prepared by Kemler [67,68].

The results of the laboratories of Franke, Jacob and Oshima identified the keratin subunits of reactive filaments in the trophectoderm of preimplantation mouse embryos and extraembryonic endoderm [66,69–73]. Subsequent cloning of the RNAs and genes for both human and mouse K8 and K18 provided tools to investigate the transcriptional regulation of these genes during early teratocarcinoma stem cell differentiation and embryo development [74–82]. However, while the dispersed regulatory elements of the K18 gene has complicated its use as an epithelial specific vector [83,84], the promoters of other IF genes have been used extensively to drive expression of genes in epidermis [85–90]. A review of the transcriptional regulation of IF genes will need to be a future opportunity.

Nestin, an IF characteristic of neuronal stem cells was discovered by Mckay's laboratory [8]. This has become a particular important marker used in human ES cell differentiation, neuronal stem cell studies and wound healing [91]. Furthermore, the relatively compact promoter has been used to generate reliable reporter genes in cells and mice [92]. Very recently, a function of nestin as a modulator of survival signaling by titration of Cdk5 has been shown [93]. Both nestin and K8/K18 may utilize the IF structure to sequester regulatory proteins and thus modulate signaling pathways.

IF and pathology

The development and widespread availability of specific IF protein antibodies permitted investigators to query different cells, tissues and states with new specific tools and resulted in a 50-fold increase in the number of annual publications on IFs from 1979 to 1989 (Figure 1). Both Franke and Weber recognized the power of these tools in clinical diagnostics [94–96]. However it was the tool of monoclonal antibodies that provided the opportunity for both biologists and pathologists to reliably identify the same antigens in different tissues or states. Early useful monoclonal antibodies were generated by the laboratories of Kemler [66] Franke and Weber [97], Lane [98,99], Ramaekers [100], Sun [101] and others. The application of these reagents permitted a degree of standardization that moved into the standard clinical pathology laboratory. The important applications of these and subsequent generations of antibodies aided in the immunocytochemical diagnosis of the tissues and tumors, an application that was recognized as early as 1979 [102]. One key characteristic of keratins and other IF that makes them useful in pathology is the relative stability of expression even after transformation to pathological states. The continued expression of characteristic keratin expression in carcinomas must reflect either an unusually stable molecular mechanism of transcriptional control or a strong selective advantage for continued expression.

While IF staining provides pathologists with tools to distinguish tumor and cell types, another application is the recognition of keratin fragments and peptides in the circulation of cancer patients. Weber and colleagues were responsible for recognizing the antigenic relationship of circulating tissue polypeptide antigen and the degradation products of simple epithelial keratins [103]. Nearly all of the antibodies found to be useful for monitoring cancers through patient sera have proven to recognize epitopes of K8, K18 and K19 [104]. The origin of keratin fragments in the circulation is the proteolytic cleavage of tumor cell keratins. The release of keratin fragments into the circulation may reflect an escape from the normally orderly disposal cell contents during apoptosis.

IF and cell death

In dying cells IFs might be considered toxic waste because misfolded proteins and protein aggregates have been implicated in many diseases such as Alzheimer's and Huntington's disease, human genetic diseases of epidermal keratins and Mallory bodies in alcoholic liver disease. The path to disposal of IF during programmed cell death was first discovered when lamins A, B and C were identified as substrates of the caspase proteases, activated by apoptosis

[105,106]. Comparison of the protein sequence of the cleavage sites of lamins to other IF revealed that K18 was a potential substrate of caspase cleavage. This prediction was verified [107]. The exact sequence of the second caspase cleavage site of K18 was also identified [108] and cleavage of the second site generates a neo-epitope for a monoclonal antibody now widely used to detect apoptotic epithelial cells[109]. The central cleavage site of K18 is within the linker region between the coiled coil 1B and 2A regions and is conserved in Type I keratins, except for those with extracellular protective functions in the epidermis (K9 and K10) and in Type III IF [110,111] and in lamins [103,104]. However, the orderly degradation of the keratin cytoskeleton is preceded by the cleavage of plectin, a cytoskeletal cross linking protein, by caspase 8 [112] and is also regulated by the adaptor protein DEDD that associates with both keratins and caspase before they are cleaved [113].

The origins of IF discovered

The similarity of different IF proteins was highlighted in 1981 by the discovery of an antigenic epitope shared by all IF proteins and recognized by a single monoclonal antibody [114]. The protein sequencing of wool keratins and desmin, and the cloning and sequencing of IF mRNAs starting in 1981 [57] revealed the conserved central coiled coil l 1A, 1B and 2A, 2B rod domains [64]. Subsequent cDNA sequences of other IFs and exon gene structure facilitated evolutionary studies. In 1986, McKeon discovered that the nuclear lamins were members of the IF superfamily but contained extended helical segments not found in other IF [115]. The extended helical segments of the nuclear lamins were found in IFs for invertebrates suggesting an evolutionary path from nuclear lamins to cytoplasmic IFs [116]. By examining the existence and structure of IF in a variety of different animals, Weber continues to provide compelling insight into evolutionary appearance, divergence and functional specialization of IFs. Identification of 11 IF genes, 5 of which are uniquely essential, in Caenorhabditis elegans [117] and 65 coding genes in the human genome [1] contrasts with the absence of cytoplasmic IF in Drosophila [25].

The function of Ifs

The exquisite IF patterns observed within cells is a reflection of the interactions of individual filaments, sometimes in dramatically regular patterns such as the tonofilament bundles found for keratins or as the spherical mesh for the nuclear lamina [118]. The great abundance of IF in certain tissues such as neuronal axons and in epidermal differentiating keratinocytes led logically to the expectation that IFs have a structural function. However, proving this idea was harder than expected. Vimentin filaments patterns could be disrupted by the microinjection of vimentin antibody [119]. However, little affect of the disruption on cell appearance or behavior was evident. Furthermore, intracellular antibody-mediated disruption of keratins did not lead to an obvious change of cell behavior [119,120]. This was a surprise, and contrasted with interfering with either actin or microtubule organization. The first disruption of IF in a multicellular embryo was obtained by injecting keratin antibody into one of two cells of the two-cell stage mouse embryo and examining the development of the blastocyst in culture [121]. Disruption of keratin filaments had no discernible effect on blastocyst formation including the keratin containing trophoblast outer layer of cells.

The first deficiency of an IF in an adult organism was reported in a spontaneous mutant quail that lacked neurofilaments. While these birds were viable, they developed neurological deficiencies including decreased axon caliber [122,123]. With the development of gene targeting methods it became possible to genetically knockout the expression of IF genes and thus eliminate the lingering concern about residual IF protein present when antibodies disrupted organization of IFs. The first IF gene to be inactivated by gene targeting technology was mouse K8 [124]. K8 deficient ES cells differentiated normally in culture to polarized yolk sac

extraembryonic endoderm. In the absence of K8 nearly all K18 also disappeared due to the rapid degradation of excess protein subunit [125]. Most K8 deficient mice died, although some could survive without K8 [126]. Embryonic edema was initially interpreted as a possible structural defect of the liver. However, many genes that affect placental function present with similar, hypoxic phenotypes [127]. The rescue of K8 deficient mice by aggregation with tetraploid embryos capable only of extra-embryonic development, proved that K8 deficient mice died due to placental deficiency, not because of embryonic defects [128]. Two interpretations of this placental defect have been proposed. Based on histological grounds, the placental hematoma formation caused by the loss of both K18 and K19 (that is equivalent to the loss of K8, the major complementary Type II subunit) was interpreted as due to trophoblast fragility [129]. Based on increased sensitivity of some K8 deficient epithelial cell lines and liver to apoptosis induced by TNF or Fas [130,131], increased sensitivity of K8 deficient placenta to activation of the maternal immune system by concanavalin A [128] and the sensitivity of K8 deficient embryo survival on maternally expressed TNF and TNFR2 [128], K8/K18 were proposed to provide protection of trophoblast cells from maternal immune system dependent, apoptotic challenges. One possible mechanisms is due to K18 titration of TRADD, a TNFR adaptor protein [132]. Recently K17 has also been implicated in resistance to TNF killing, perhaps by sequestering TRADD, an adaptor protein, for the TNF R1 and also down regulating Flip, an inhibitor of caspase 8 [133]. Post-translational regulation of Flip, was first reported in K8 deficient mice [134] but was not confirmed with different antibodies [135]. A protective role of K8 was also revealed in liver disease [136]. Omary later showed that K18 mutations were a risk factor for liver disease in humans [137]. The increased sensitivity of K8 deficient hepatocytes to manipulation [138] and the fragility of hepatocytes over expressing a mutant K18 [139] were interpreted as reflections of structural functions for K8/K18. It is possible that these filament proteins may have both structural and regulatory functions.

The survival of K8 deficient mice to birth was dependent on modifier genes that differ between C57Bl6 and FVB/N mice. Only about half of the expected K8 null mice died before birth in the FVB/N background. Thus K8 is important, but not absolutely essential for placental function. Again this was a surprising result as K8 was the only type II keratin of hepatocytes and was the dominant component in pancreas. However, just like the neurofilament deficient quail, K8 deficient mice proved not to be completely “normal”, as closer inspection revealed bacteria-dependent colonic hyperplasia [140,141], hypersensitivity of the liver to stress [135, 136,138] and alterations in intestinal epithelial membrane proteins [142] [143].

Soon joining the ‘escaper” K8 deficient mice, were viable mice deficient in vimentin [144], desmin [145] [146], GFAP [147], K19 [148], NF-L [149], NF-M [150] and NF-H [40,41, 151]. However, while all of these IF deficient mice were viable, all also revealed phenotypes found either by testing during an appropriate condition or in combination with other deficiencies. For example, desmin deficient mice developed muscle and cardiac defects [145, 146] that were due to decreased myofibril strength [152]. Desmin mutations causing similar conditions in humans were found in 2000 [153]. Vimentin deficient mice were apparently normal [144] but defects in wound healing [154] and endothelial cell stability have recently been discovered [155].

The subtle phenotypes of some single IF knockout mice were due to compensatory functions of other family members. The combination of vimentin deficiency and GFAP deficiency revealed alterations in response to brain injury [156]. In the case of the simple epithelial keratins, both K18 and K19 deficient mice are viable but the combination is lethal [129]. Similarly the combination of K8 and K19 deficiencies increased the penetrance and decreased the time before lethality [148], apparently due to partial protection by low levels of K7 and its preferential polymerization with K18 [157]. Recently, the regulatory role of K17 in cell size [158], K8 phosphorylation in susceptibility to apoptosis [135], and the importance of NF-M

in neurons [19] are a few examples of the functions of IF that were not immediately evident in knockout mice. One illustrative test of similar functions for individual members of the keratin family was the attempt to complement K14 deficiency with K18 [159]. The failure of K18 to complement K14 deficiency suggests a different, or at least specialized functions of K18 and K14 just as ectopic expression of desmin filaments in K5 deficient animals failed to complement the skin defects [160]. The specific functions of each of the different types of intermediate filaments are discussed in the accompanying articles. However, clearly, from a historical perspective, expectations that IFs would have similar functions in different tissues, or that IFs would have universal functions, analogous to cellular functions of microfilaments or microtubules required re-evaluation.

The function of nuclear lamins is an example of IF with both structural and regulatory functions. While lamin B1 and/or B2 composed the nuclear lamina of all mammalian cells and are essential for viability [161] mutations in the LMNA gene that generates lamins A and C are responsible for a great variety and number of genetic diseases [16,24]. Nuclei of lamin A deficient or mutant cells have nuclear structure alterations, increased fragility and decreased mechanical stiffness [162–164] but mutations in lamin A also result in alterations in tissue specific gene expression most likely due to the association of the nuclear lamina with chromatin and specific transcription factors [165–167]. Lamin A/C may be important for the perinuculear location of genes that facilitates gene silencing. [168]. The C-terminal, non alpha-helical region of lamin has multiple interaction domains including a terminal, specialized membrane attachment region. Muations that interfere with the processing of the farneslylated CAAX-box cause premature aging, the Hutchison-Gilford progeria syndrome. Thus specialized functions of lamin A reside in the unique domains flanking the helical domains shared with other IF proteins.

If individual IFs have unique functions, filament organization disruption strategies may have different outcomes. For example, the binding of proteins such as Cdk5 [93], Jnk [169] or 14-3-3 [170,171] to IF might still occur in cells with disorganized filaments but not in cells with none of the protein. However, disorganization of filament organization that primarily provides structural integrity may cause structural failure or cytolysis as occurs with disruption of K14 or K10. Over expression studies of specific mutant protein forms have been revealing in the study of IF regulatory functions, for example, in the titration of regulatory molecules by competitive binding [135].

Human disease and intermediate filaments

The discovery of human diseases that are caused by mutations in IF genes proved the relevance of the basic research efforts and provided evidence for structural functions for epidermal keratins. The first disease of IFs was epidermolysis bullosa simplex (EBS), a rare genetic skin disease characterized by blistering of the skin. The blisters arise due to cytolysis of the basal layer of cells that have disorganized keratin tonofilaments, beneath the more differentiated skin cells. The identification of EBS patients with point mutations in the K14 [59] and K5 [172] genes was preceded by the results of expressing normal and mutant K14 in transgenic mice [173,174] that had striking resemblance to the human disease. This disease was also observed in a patient with a complete absence of K14 [175], equivalent to a targeted gene knockout. Mutations within keratin 1 and 10 were discovered as the basis of another genetic disease, epidermolytic hyperkeratosis that led to fragility in the upper layers of the skin [176–178]. Subsequently disease-causing or associated mutations have been described in most IF genes of all five classes [16].

More recently laminopathies composed of at least 12 different diseases have been defined by over a hundred individual mutations of the LMNA gene [24,179]. These diseases affect muscle,

adipose, bone, nerve and skin and even the aging process and have provided an entree for understanding the underlying molecular interactions of lamins. Furthermore, the dispensability of lamin A, when lamin C is present provides some hope for treatment by suppression of mutant lamin A expression. [180]. When combined with the near universal use of IF antibodies for diagnosis and the expanding use in stem cell research, it is evident that research investment in intermediate filaments has yielded an excellent return in both health impact, conceptual expansion and independent investigators (Figure 2).

Back to the Future

What does the future of IF research hold?

An important question is the pathological consequences of IF protein aggregates. Intracellular inclusions of IF proteins are associated with IF diseases of the liver, muscle and brain. While Mallory body formation may not be necessary for increased toxicity of certain liver disease models [136], protein aggregation and/or precipitation has been implicated in many different human diseases. Even an imbalance of expression of complementary keratin subunits may place a heavy challenge on a cells ability to degrade and dispose of dangerously insoluble proteins. The responses of cells to such material may sensitize them to additional stress. Understanding the contributions of direct functions, such as titrating regulatory proteins or providing strength and secondary responses to imbalanced or aggregated IF proteins will be key to understanding IF associated disease states.

The structure of IFs at atomic resolution still remains to be solved and the determinants of multi-filament organization and branching are of great interest. The roles of IFs in modulating intracellular signaling, both apoptotic and kinase stimulated pathways will likely lead to molecular explanations of additional roles. Finally, the molecular basis of the tissue specific transcriptional regulation of many IF genes remains to be determined. For example, the simple epithelial keratins K8, K18 and K19 were isolated as differentiation markers of pre-implantation embryos and teratocarcinoma stem cells. Human ES cells like human embryonal carcinoma cells [181] and unlike mouse ES cells, express abundant keratin filament RNAs and proteins. (data not shown). The transcriptional regulatory determinants of the differences of mouse and human ES cells are not known.

Great progress in both associating IF gene mutations with human disease and discovering non-structural functions of specific IFs appears to have stimulated renewed interest and potential for an exciting future.

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Abbreviations

GFAP
glial gibrillary acidic protein

NF
neurofilament

NF-L
neurofilament-low molecular mass protein

NF-M
neurofilament-medium molecular mass protein

NF-H
neurofilament-high molecular mass protein

IF
: intermediate filament

ALS
: amyotrophic lateral sclerosis

ES
: embryonic stem

Cdk5
: cyclin-dependent kinase 5

DEDD
: death effector domain containing

TRADD
: tumor necrosis factor receptor type 1-associated death domain protein

TNFR
: tumor necrosis factor receptor

LMNA
: lamin A gene

EBS
: epidermolysis bullosa simplex

Flip
: Flice-like inhibitory protein

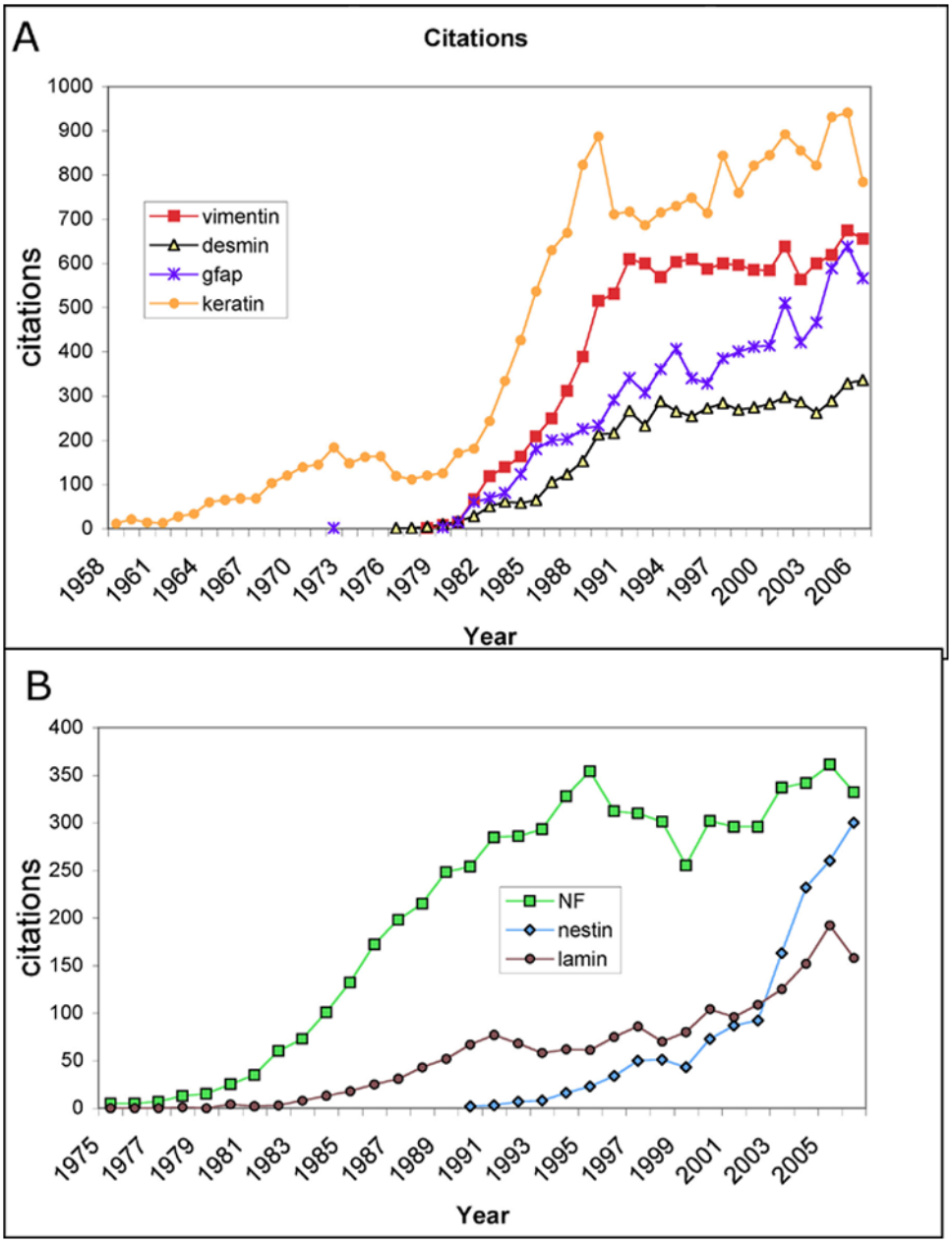

Figure 1.
Intermediate filament publications. The PubMed database was searched with the indicated key word and year. A, keratin, vimentin, desmin and glial fibrillary acidic protein (GFAP). B, nestin, neurofilament forms (NF) and lamin citations are shown.

Figure 2. Participants of a research conference on intermediate filaments, July 1992

1 R. Liem; 2 K. Green; 3 R. Linck; 4 H. Pant; 5 I. Carey; 6 B. McCormick; 7 R. Miller; 8 M. Kiurpakus; 9 K. Albers; 10 P. Opal; 11 J. Erikson; 12 R. Evans; 13 O. Skalli; 14 P. Hornbeck; 15 D. Sun; 16 T. Letai; 17 T. Hashimoto; 18 s. Chin; 19 Y. Chan; 20 S.C. Lee; 21 T. Frappier; 22 C. Sommers; 23 M.K. Lee; 24 R. Presland; 25 M. Monteiro; 26 Z. Xu; 27 J. Rothnagel; 28 E. Glasgow; 29 B. Lu; 30 M. Schecter; 31 M Carden; 32 a. Eckect; 33 B. Druger; 34 H. Herrmann; 35 J. Vickers; 36 A. Trejo; 37 I. Nicholl; 38 E. Rugg; 39 K. Straube; 40 J. Eyer; 41 H. Baribault; 42 U. Aebi; 43 R. Moir; 44 J.M. Navarro; 45 T. Stappenbeck; 46 J.M. Paramio; 47 J. harris; 48 J. Cholberg; 49 R. J. Williams; 50 R. Wu; 51 P. Coulombe; 52 M. Manabe; 53 J.s. Gordon; 54 M. O'Guin; 55 S.Y. Kim; 56 L. Hutton; 57 L. Marekov; 58 Y. Captetanki; 59 J.M. Yang; 60 A. Goldman; 61 D. Paulin; 62 F. Gounari; 63 Y.H. Chou; 64 W. Geraros; 65 J. Nash; 66 W.J. Chen; 67 N. Stuurman; 68 M.M. Portier; 69 F. Landon; 70 K. Yoneda; 71 J Compton; 72 I.G. Kim; 73 D. Mischke; 74 B. Korge; 75 D. Parry; 76 H. Boemendal; 77 M. Sanders; 78 S.S. Lim; 79 G. Ghing; 80 C. CChien; 81 M. Hatzfeld; 82 I. Freedbery; 83 M. Klymkowsky; 84 J. Jones; 85 E. Gardner; 86 K. Laur; 87 A. Steven; 88 P. Steinert; 89 P. Hoffman; 90 N. Jorplawa; 91 T. Belecky-Adams; 92 L. Parysek; 93 G. Wiche; 94 E. Nigg; 95 N. Markova; 96 T. Trevor; 97 B. Lane; 98 D. Roop; 99 B. Dale; 100 I. Makarova; 101 R. Goldman; 102 E. Fuchs; 103 S. Khan; 104 D. Cleveland; 105 W. Ip; 106 R. Oshima; 107 F. McKeon; 108 Z. Zehner; 109 T. Sun; 110 G. Shaw; 111 R. Quinlan; 112 E. White; 113 F. Van de Kluder; 114 S. Georgatos.

Table 1

Some Landmark IF Publications

Class	Members	Examples[a]	Identification/ Isolation[c]	Sequence[d]	KO/Mutation[e]
1	K9–K20	K10	1982 [61] [101]	1984 [182]	1992 [176]
		K14	1978 [54]	1982 [58]	1991 [59]
		K18	1980 [69]	1986 [75]	1998 [157]
		K19	1981 [66]	1989 [183]	2000 [148]
	K23–K28		[184]		
	K31–K40[b]		[184]		
2	K1–K8	K1	1982 [61] [101]	1983 [185]	1992 [176]
		K5	1978 [54]	1984 [186]	1992 [172]
		K8	1980 [69]	1985 [77]	1991 [124]
	K71–K80		[184]		
	K81–K86[b]	K86	[184]		1997 [187]
3	vimentin		1978 [50]	1983 [188]	1994 [144]
	GFAP		1972 [5]	1984 [189]	1996 [146]
	desmin		1976 [4]	1982 [64]	1996 [146] [145]
	peripherin			1983 [6]	
4	NF-L		1978 [52]	1985 [190]	1997 [149]
	NF-M		1978 [52]	1986 [104]	1991, 1998 [122] [150]
	NF-H		1978 [52]	1986 [104]	1998 [39] [40]
	synemin		1980 [191]	1995 [192]	
	a-internexin		1985 [193]	1990 [194]	1999 [195]
	nestin			1990 [8]	
	syncoilin			2001 [10]	
5	Lamin A/C		1978 [196]	1986 [115]	1997 [163]
	lamin B1		1978 [196]	1987 [197]	2001 [161]
	lamin B2			1988 [198]	
other	filensin			1991 [199]	2003 [200]
	phakinin			1993 [201]	2002 [199]

[a] Only a few pertinent examples of the keratin classes are shown for brevity.

[b] Hair and root sheath keratins are not included because of space. Pleaser refer to [183] as a starting place for references on these genes.

[c] In most cases these refer to the date of isolation of the indicated protein or definitive identification of unique species.

[d] The primary amino acid sequence of the protein, determined directly or deduced from the cDNA.

[e] Year of publication of the description of either the mouse knockout or the animal or human mutation first associated with the disease.